

The University of Chicago

EMBRYO ABORTION IN EARLY-
RIPENING VARIETIES OF
PRUNUS AVIUM

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES FOR THE DE-
GREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

1932

By

HAROLD BRADFORD TUKEY

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THE BOTANICAL GAZETTE

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EMBRYO ABORTION IN EARLY-RIPENING VARIETIES OF PRUNUS AVIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 439

H. B. TUKEY

(WITH FORTY-ONE FIGURES)

Introduction

The failure of early-ripening varieties of stone fruits, mainly of varieties of *Prunus*, to mature viable seed has been noted by both nurserymen and plant breeders. Nurserymen fail to secure good stands of seedling understocks of peach, mazzard cherry (*Prunus avium* L.), and mahaleb cherry (*P. mahaleb* L.) when the seed supply is obtained from wild trees which produce early-ripening fruit. Plant breeders find that crosses in which an early-ripening variety is used as the female parent produce viable seed only rarely.

BLAKE (1) pointed out that the flesh of some varieties of peaches ripens before the seed matures. MARSHALL (12) divided varieties of plum (*P. domestica* L.) into two groups, namely, those bearing mature fruits each of which contains a viable seed and those bearing mature fruits only a few of which contain viable seed. CHANDLER (3) reported never finding kernels in such early varieties of peach as the Sneed, although pollination is necessary for fruit production. CONNORS (4) stated that varieties of peaches ripening their fruit 75-80 days after pollination do not develop viable seed in New Jersey; that those which ripen 85-90 days after full bloom develop 15 per cent viable seed; and that later-ripening varieties produce a good

proportion of viable seeds. WELLINGTON (21) reported that records of cherry crosses indicated that early-ripening varieties used as parents had few viable seeds, medium-ripening sorts a higher percentage, and very late-ripening kinds a still higher percentage. TUKEY (18) showed that with 17 varieties and types of sweet cherry and 11 varieties and types of sour cherry, those kinds that were late-ripening (that is, requiring 80 days from full bloom to fruit ripening) developed nearly 100 per cent viable seed; those that were early-ripening (requiring 60 days or less from flower bloom to fruit ripening) developed almost no viable seed; and the mid-season sorts (those requiring 65-75 days to mature fruit) developed an intermediate number. SAKAGUCHI (17) recommends that plant breeders do not use as female parents peach varieties which ripen the fruit in fewer than 100 days after full bloom.

The problem presented by these facts suggests a critical comparison of the embryogeny and fruit development of early and late-ripening varieties of sweet cherry (*P. avium*). Furthermore, although critical studies have been made of both the early and the late stages in seed and fruit development, there is a dearth of material for the stages intervening. This paper attempts to supply information regarding these two phases of the subject.

Various phases in the embryogeny of the cherry have been reported by BRADBURY (2), MALPIGHI (11), PÉCHOUTRE (15), RUEHLE (16), TULASNE (20), WENT (22), and others. Studies in related genera which show similarity in development of related parts have been made by DORSEY (6) and OSTERWALDER (14). The growth of fruits of related species of *Prunus* has been studied by BLAKE (1), CONNORS (4), DORSEY and McMUNN (7), LILLELAND (10), and SAKAGUCHI (17). A more detailed mention of these findings will be made with reference to the particular point involved.

Procedure

Fresh material was dissected under the binocular microscope. This method provided a rapid means of securing an adequate number of measurements of the various stages of development. In addition, it proved useful in helping to interpret the prepared slides.

Flower and fruit parts from twigs placed in water and held at

2°C. for 72 hours showed no measurable development, thus permitting the gross examination of a great number of individuals over a comparatively long period of time at identical stages of arrested development.

Material from open-pollinated blossoms in a mixed varietal orchard was most satisfactory for general study because of its more uniform rate of development. For comparison with the open-pollinated blossoms, however, blossoms were emasculated before they had opened and were pollinated by hand 3 days later, when the stigmas were receptive. Pollen was used which had been removed in the laboratory from closed blossoms and ripened at a temperature of 25°–30°C. Emasculated blossoms were kept covered with tight paper bags from the time of emasculation until the fruit had set. The paper bags were then replaced by cheese-cloth bags. Open-pollinated, self-pollinated, and cross-pollinated material was prepared, the crosses being between known compatible varieties and the pollen being tested by germination at room temperature (20°–25° C.) in 5 per cent sucrose solution.

Pollen tubes were traced in the carpel under the dissecting microscope from fresh material crushed in lacmoid-martius-yellow after the method described by NEBEL (13). The varieties used for critical study were Early Purple Guigne, Burbank, Lyons, and Black Tartarian, representing early-ripening kinds; Eagle, representing a mid-season sort; and Windsor, Lambert, Downer, and Mazzard, representing late-season varieties. Others examined for comparison were Governor Wood, Knight's Early Black, Elton, Napoleon, Schmidt, Black Republican, Abundance, and Oswego. The work was conducted at Geneva, New York, throughout the growing seasons of 1930, 1931, and 1932. Records for the years 1926 to 1929 inclusive have been used to corroborate the findings from the more exact studies of 1930 to 1932 inclusive.

Material for slides made after the paraffin method was killed and fixed in two solutions, designated solution I and solution II. Solution I was made up of 100 cc. of 50 per cent alcohol, 2.3 cc. glacial acetic acid, and 6.7 cc. formalin; solution II (Karpechenko's chromacetic) was made up of two parts, part A consisting of 1 per cent chromic acid in water and part B consisting of 10 cc. of glacial acetic

acid and 40 cc. of formalin. The two solutions were kept separate until used for fixation, when two parts of A by volume and one part of B were poured together. The material was first placed in solution I while being prepared, then placed in solution II, the whole being then exhausted to 600 mm. of mercury in a desiccator, and held at 4° C. for 24 hours. Excellent fixation was secured by this method, the material being equally as good for cytological as for histological studies. In the case of ovaries 6–12 mm. in length, longitudinal slices were cut from both sides of the ovary, parallel to the sutures and including portions of the nucellus, thus permitting rapid penetration. With larger material the seed was removed from the carpel.

Safranin, orange G, and crystal violet were used for staining.

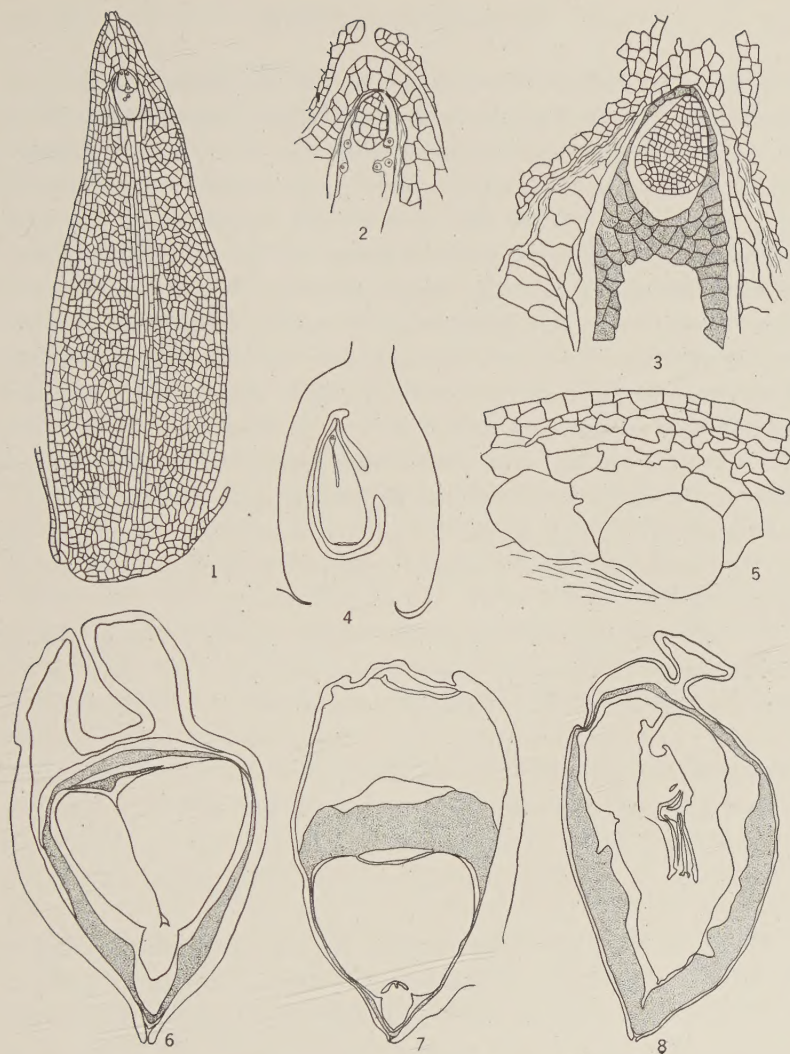
Measurements were made with an eyepiece micrometer, both sliding and fixed, and checked against each other. Care was necessary to secure median measurements and to secure fruits of average development. In the compilation of data shown in the tables, the mean of from three to seventeen individuals is represented for each measurement. The development of individuals collected at the same time showed very little variation, so that a smaller number would have served equally as well. Measurements from prepared slides were checked against those from fresh material dissected under the binocular microscope.

Normal development

Although certain stages and periods of development of particular parts of the cherry seed and fruit have been studied by other workers, no systematic record of development has been made, nor has any attempt been made to correlate the development of various parts. Accordingly the steps are here presented, and for this purpose the Downer variety of sweet cherry has been selected as typical or "normal." It is a late-ripening sort which normally produces a high proportion of viable seed.

1. OVARY.—The ovary increases in length only slightly from the 55th to the 14th day before full bloom, namely, from 0.54 mm. to 0.74 mm. At this time it begins a more rapid period of enlargement, until at the time of full bloom it is 2 mm. in length.

At the time of fertilization, a day or two after full bloom, there is



FIGS. 1-8.—Fig. 1, nucellus and 7-celled megagametophyte 2 days before full bloom, showing central core of conductive tissue of elongated cells from chalaza to megagametophyte through which the megagametophyte and embryo-sac develop. Fig. 2, young embryo and free-nuclear endosperm 5 days after full bloom. Fig. 3, young embryo and cellular development of endosperm 12 days after full bloom. Fig. 4, development of megagametophyte, nucellus and integuments, and ovary from an emasculated, unpollinated blossom, 7 days after full bloom. Fig. 5, endosperm layer at chalazal end of seed, 37 days after bloom, showing growth by cell divisions in peripheral layer, region of cell enlargement, and region of digestion by adjacent advancing embryo. Figs. 6, 7, typical aborted seeds of Early Purple Guigne, 27 days after full bloom, showing peculiar flattening of cotyledons. Fig. 8, unusual embryo development in abortive seed, bearing 6 true leaves.

a sudden acceleration in the development of the pericarp, which continues for a period of 17 days after full bloom. During the season of 1931 the ovary increased in length from 2.46 mm. the 1st day after bloom to 3.31 mm. on the 4th, 4.38 mm. on the 6th, 7.5 mm. on the 13th, and to 10.5 mm. on the 17th. This is in contrast to the small increase prior to the time of fertilization. At the end of this period, development of the pericarp ceases almost as abruptly as it began, and a period of greatly lessened growth ensues, which in 1931 extended from the 17th to the 31st day, or a total of 14 days. A second period of rapid pericarp development follows the period of retarded development, continuing until the fruit is dead ripe, or from the 31st to the 66th day after full bloom during the season of 1931. Similar periods of accelerated and retarded pericarp development have been reported by BLAKE (1) and by DORSEY and McMUNN (7) for the peach, and by LILLELAND (10) for the apricot (*Prunus armeniaca* L.).

2. NUCELLUS AND INTEGUMENTS.—There are two ovules, arising from opposite placentae of the single carpel, only one of which develops to maturity. The micropyle points toward the distal end of the ovary and the micropylar end of the ovule lies against an outgrowth of the placenta, the obturator (RUEHLE 16). Both ovules develop equally but slowly up to 14 days before full bloom, paralleling the development of the ovary. They then begin a more rapid enlargement and double in length by full bloom unless arrested in development.

At the central axis of the nucellus, reaching from the chalaza to the base of the megaspore mother cell or of the megagametophyte, is found a core of elongated cells as shown in figure 1, which take gentian violet stain readily and split apart readily in a longitudinal direction. The surrounding cells of the nucellus are more nearly isodiametric, thin-walled, with less dense cytoplasm. The chalazal region is clearly marked as a deeply staining tissue.

Two to 7 days before full bloom one of the ovules is abruptly arrested in development, as mentioned by RUEHLE, but does not shrivel and collapse until 3 or 4 days after full bloom. In some varieties, as Lyons, it is common for both ovules to develop until the period of embryo abortion.

At the time of fertilization there is a sudden acceleration in growth of the nucellus and integuments. By 17 days after bloom they have reached 5.71 mm. in size, compared with a maximum of 7.0 for Downer during the season of 1931. From this date until the fruit is nearly ripe there is a slight gain in size, and then a small decrease as the integuments shrink slightly at about the time of fruit ripening.

TULASNE (20) has remarked upon this early attainment of maximum size by the ovules of *Prunus spinosa* L., *P. avium*, and *P. mahaleb*, the ovule of the first reaching a length of 5 mm. before fertilization. PÉCHOUTRE, too, mentions that the ovule of *P. cerasus* L. reaches almost full size at a very early stage of fruit development.

3. MEGAGAMETOPHYTE AND EMBRYO SAC.—The stages in megaspore formation and megagametophyte development have been studied by RUEHLE (16) for the sweet cherry, by BRADBURY (2) for the sour cherry (*P. cerasus*), for several species of *Prunus* by PÉCHOUTRE (15), and by others. The development as carefully pictured and described by BRADBURY for the sour cherry seems identical with the development of the sweet cherry, so that a critical comparison would be superfluous. Accordingly only the time relationship will be emphasized.

The megaspore mother cell is first distinguished 14 days before full bloom, at the time the individual flower buds have separated in the cluster. It lies beneath five to eight rows of parietal cells, made up of the "coiffe épidermique" and the "calotte" of PÉCHOUTRE. It may remain in this stage for several days before reduction division. Following this division, disintegration of the three distal megaspores in the linear tetrad occurs rapidly. Thus the stages most commonly observed are either that of the megaspore mother cell or of the functional megaspore.

The megagametophyte is fully developed during the following 7 days, or 3 to 7 days before full bloom, at about the time the petals of the blossoms are showing. Development to the 7-celled stage is rapid. The polar nuclei, however, do not migrate to the center of the megagametophyte until about the time of full bloom, and then fuse a day or two prior to fertilization. A few starch grains are found in the megagametophyte at this time.

The synergids persist for some time, even 5 to 10 days after fer-

tilization. On the other hand, the antipodals disintegrate almost immediately after they are formed. The megagamete is relatively small and remains close to the micropylar end of the megagametophyte.

In the case of the non-functional ovule, which is commonly arrested in development just before full bloom, the megagametophyte may persist unelongated but otherwise complete for 10 to 14 days longer. Even after the nucellar tissue has collapsed, the integuments have shriveled, and degeneration has begun in these parts, the nuclei of the megagametophyte and of the megagamete may remain apparently unchanged, although arrested in growth.

At the time of fertilization there is a rapid elongation of the megagametophyte inclosed by the megaspore membrane. During the season of 1931 this increase in length was from 0.67 mm. 4 days after full bloom to 4.73 mm. 13 days later. It passes easily through the central core of elongated cells (fig. 1), as though in response to turgor pressure within, to assume a characteristically long tubular shape noted as long ago as 1675 by MALPIGHI (11), and called by him "vas umbilicale" and "umbilicus." Such acceleration is even more rapid than the growth of either the ovary or of the nucellus and integuments. It continues for a period of 17 days after full bloom, at which time the embryo sac, inclosed by the megaspore membrane, extends nearly to the chalaza. Development may begin 24 hours before pollen tubes can be found within the ovary, and therefore before fertilization has been accomplished; or it may be delayed until 4 or 5 days after full bloom, at which time a very small embryo can be distinguished.

This rapid development of the megagametophyte and embryo sac has been described by TULASNE for several species of *Prunus*, including *P. avium*, by PÉCHOUTRE who called it a "canal," and by WENT.

Occasionally the chalazal end swells to assume a balloon-like shape, and usually there is more or less narrowing about midway, similar to that described by WENT as an isthmus. WENT also pictures the antipodals as persistent at the chalazal end of the elongated embryo sac. This situation has not been observed in these studies. There is, however, a characteristically large endosperm nucleus

present at the tip of the elongating embryo sac at the chalazal end, which gives the embryo sac a pointed appearance.

Representation of the embryo sac by some investigators as a much-branched haustorial organ ramifying the nucellar tissue near the chalazal end, or as a much-enlarged balloon-shaped lacuna, is probably due to swelling and to artifacts occasionally produced in fixation. Such conditions are not found in fresh material.

The megaspore membrane, which is the outer layer of the embryo sac, is continuous and persistent. With dissecting needles it can be removed intact from fresh material, and still later in development the embryo, cellular endosperm, and embryo sac containing free-nuclear endosperm may all be removed together as though surrounded by the same continuous membrane. TULASNE (20) has also remarked upon the persistence of this membrane in dissection. The last vestiges of it may still be seen pressed against the chalazal portion of the nucellus by the developing endosperm 36 days after bloom.

The haustorial nature of the embryo sac will be discussed in connection with endosperm development.

4. FERTILIZATION.—Fertilization is as pictured for the sour cherry by BRADBURY (2). The sweet cherry, however, is self-sterile. In the case of emasculated flowers, hand-pollinated with tested pollen of known compatible varieties, pollen tubes had penetrated the style only slightly 24 hours after pollination. They were traced to the base of the style 48 hours after pollination in selfed flowers, but no further. In the case of cross-pollinated flowers, pollen tubes were found within the ovary, and occasionally within the micropyle at this time. In all open-pollinated blossoms, numerous pollen tubes could be found traversing the style, penetrating the ovary, and within the micropyle. The effect of emasculation and bagging is to retard the development of all parts from 4 to 6 days but otherwise to produce no unfavorable results.

5. ENDOSPERM.—Both the embryo and the endosperm develop very slowly for a considerable time after fertilization. The primary endosperm nucleus divides prior to the division of the zygote as described by BRADBURY for the sour cherry, and by PÉCHOUTRE.

It remains free-nuclear for some time (fig. 2). The nuclei of the chalazal region are large and relatively far apart. This portion never becomes multicellular. The nuclei of the micropylar region are smaller, close together, and this portion subsequently becomes cellular.

In *Pyrus*, the embryogeny of which is similar to that of *Prunus*, it has been shown by OSTERWALDER (14) that one of the first two endosperm nuclei migrates toward the micropylar end of the embryo sac and is the nucleus from which the cellular endosperm is subsequently derived; while from the other is formed the large-nuclear endosperm of the chalazal region. That this is probably the situation in *Prunus* is suggested by the material.

WENT has described the isthmus of constriction which divides the embryo sac midway into regions. PÉCHOUTRE discusses the elongate embryo sac containing free-nuclear endosperm from the standpoint of rapid movement of food materials from the chalazal region of the nucellus to the embryo and cellular endosperm in the micropylar end of the embryo sac. RUEHLE considers the embryo sac as a haustorial organ, and traces the movement of food materials from the vascular bundles of the integuments through the cells of the chalazal region and through the elongate embryo sac. OSTERWALDER considers the embryo sac of *Pyrus* as serving a haustorial purpose. He suggests that the difference in the shape and size of the endosperm nuclei at opposite ends of the embryo sac is due to nutritive conditions. He also suggests that cell walls are not laid down beyond the isthmus toward the chalazal region because of the chemical make-up and nutritive function of that region.

Cell walls begin to appear the 9th day after full bloom between the nuclei surrounding the embryo at the micropylar end of the embryo sac. Cell wall formation subsequently continues slowly until the 17th day after full bloom, the cellular endosperm reaching a length of only 0.57 mm. at this time during the season of 1931. The cell walls are formed progressively from the micropylar to the chalazal end, and from the periphery inward, much like the deposition of layers of material upon the inside of a pear-shaped bag until it becomes a solid mass (fig. 3). At the region of the isthmus reported by WENT cell walls are sometimes formed, and then often with sev-

eral nuclei in one cell. In the region of the chalaza no cell walls are formed. The increase of the endosperm after it has formed this cellular mass is still from the micropylar toward the chalazal end, but is by cell division rather than by the addition of cell walls to free-nuclear endosperm.

This period of delayed development is followed by a period of very rapid enlargement in which the cellular endosperm increases from 0.57 to 4.8 mm. between the 17th and 31st day after full bloom. There is an active region of cell division at the periphery of the cellular mass of endosperm (fig. 5) away from the micropylar end. The newly formed cells enlarge and are in turn digested by the advancing embryo. There is no indication of the endosperm being "pushed ahead" of the developing embryo, as has been stated in the literature. In the thinner portions of the endosperm it may be only 6-8 layers of cells thick. At maturity, the seed is entirely surrounded by a thin layer of endosperm 0.15 mm. thick at the chalazal end.

6. EMBRYO.—The first division of the zygote is to form a large suspensor cell. The suspensor is small, as described by PÉCHOUTRE and RUEHLE, and similar to that described for the sour cherry by BRADBURY. It is persistent to maturity of the embryo. The development of the embryo is even slower than that of the endosperm. Four days after full bloom in 1931 the embryo had passed the zygote stage, but from then until the 24th day after full bloom it had reached a total length of only 0.16 mm. In the early stages of embryo development, the embryo sac contains many tiny starch grains as reported by BRADBURY.

At this time there occurs a sudden rapid development so that the embryo increases in length from 0.71 mm. on the 27th day after full bloom up to 3.00 mm. on the 31st day. Material killed and fixed during this period shows numerous mitotic figures of various stages throughout the embryo. Development from this point is regular but less rapid. The end result is an embryo with two well developed cotyledons, surrounded by a thin layer of endosperm and a fine line of nucellar tissue, the whole completely filling the integuments.

DORSEY and MCMUNN have noted a similar slow, early development of the embryo in the peach (*Prunus persica*), followed by very rapid enlargement. They give the length of the young embryo in the

Elberta peach as only $1/16$ of an inch two months after full bloom, and then describe a period of rapid development for one month.

7. EMBRYO ABORTION.—When embryo abortion occurs it is observed as a sudden arrest in development of the embryo. The nucel-

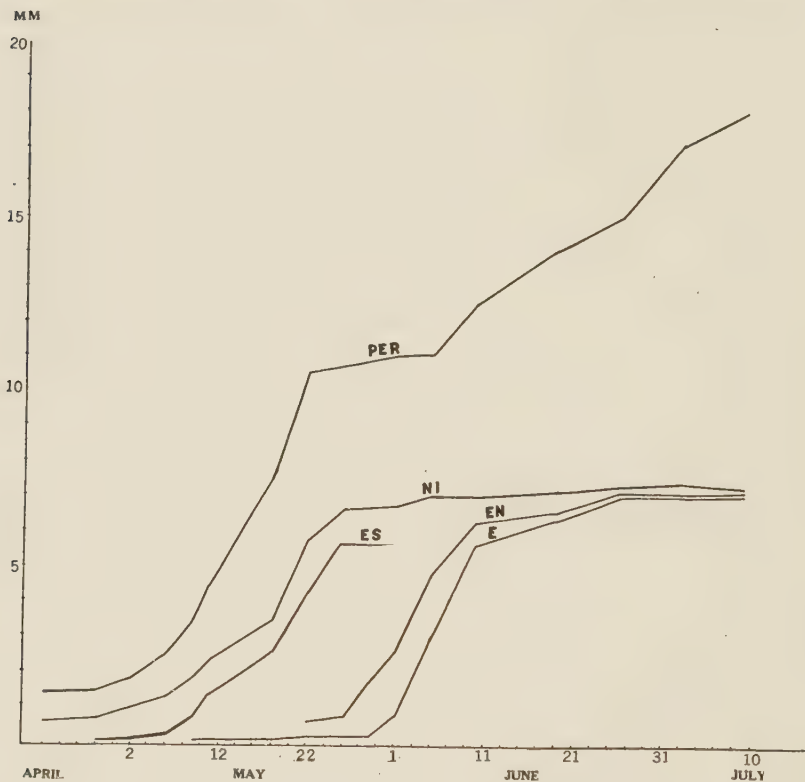


FIG. 9.—Graph of typical development of pericarp (*per*), nucellus and integuments (*ni*), megagametophyte and embryo sac (*es*), endosperm (*en*), and embryo (*e*) of a late-ripening variety of sweet cherry (Downer), which produces a high proportion of viable seed.

lar tissue collapses, the integuments shrivel, and the characteristic aborted seed shown in figures 6 and 7 is the result. The aborted embryo may begin to disintegrate 5 days after arrest in development, or it may remain intact for at least 42 days. There is great variation in the stage of development of the fruit and in the stage of development of the embryo at which abortion occurs.

Critical examination of embryos of dropped fruits from the sweet cherry variety normally producing viable seed has shown no apparent structural variation from a developing embryo of a developing fruit. Neither does there appear to be any structural variation in the

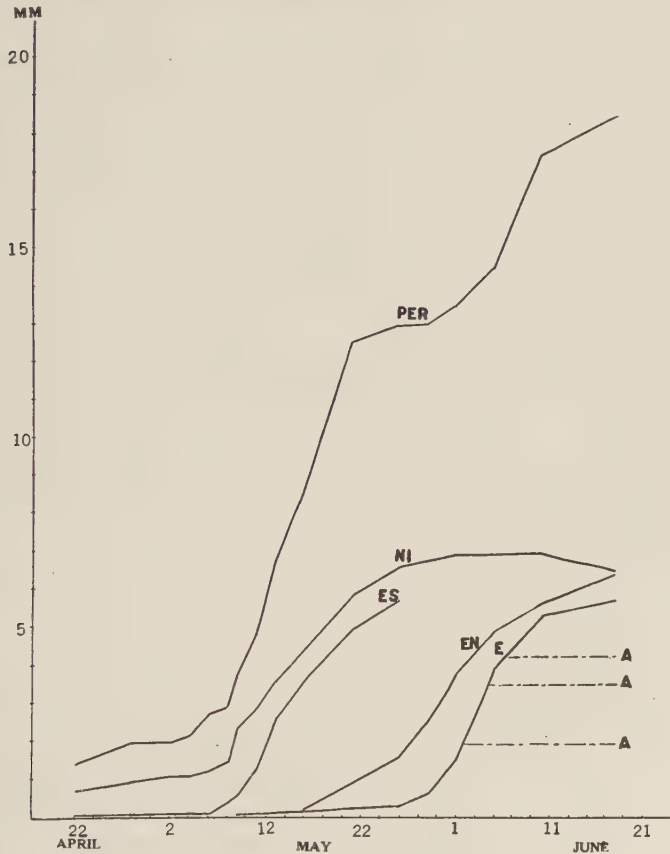


FIG. 10.—Graph of typical development of pericarp, nucellus and integuments, megagametophyte and embryo sac, endosperm, and embryo of an early-ripening variety of sweet cherry (Early Purple Guigne), which rarely produces viable seed.

nucellus or the endosperm of ovules containing abortive seed, with the exception of a suggestion of collapse of nucellar tissue adjacent to the developing cellular endosperm. On the other hand, there is a tendency for the embryo in dropped fruits to continue development,

as reported by BRADBURY. Figure 8 shows an unusual development of an abortive embryo in a dropped fruit. The embryo has "germinated" and has developed six true leaves.

Usually embryo abortion is associated with dropping of the fruit. BRADBURY has shown the various waves of fruit drop for the Montmorency sour cherry and has explained them as resulting from factors of nutrition. KRAUS (8) has pointed out that most fallen fruits contain embryos in some stage of development. DETJEN (5), working with the apple, pear, and plum, has reported that most of the fruits of the June-drop stage contain embryos or tissues resulting from fertilization. He also points out, however, that there is a varietal character involved in the dropping of fruits; that is, trees of the same variety under identical conditions react similarly whereas trees of unlike varieties react differently.

On the other hand, embryo abortion may also be a characteristic of mature fruits of certain varieties. Mention has been made earlier in this paper of the findings in this regard of MARSHALL (12) for the plum; BLAKE (1), CHANDLER (3), CONNORS (4), and SAKAGUCHI (17) for the peach; and WELLINGTON (21) and TUKEY (18) for the cherry.

8. CORRELATION OF DEVELOPMENT.—The development of the various parts discussed in the preceding pages in relation to one another is shown graphically in figure 9. Figures 11 to 25 present camera lucida drawings of development at given stages.

In brief, the ovary and ovule parallel each other in development during the early stages. They develop only slightly from the 55th to the 14th day before full bloom. Between then and the time of full bloom the ovary and the nucellus and integuments begin a more rapid enlargement. The megaspore mother cell is formed at the beginning of this period, and the megagametophyte is in turn formed from it 3–7 days before full bloom. One of the two ovules is arrested in development just before full bloom, and the other takes the ascendancy.

At the time of fertilization there is a sudden acceleration in growth of the ovary and of the nucellus and integuments, and a marked elongation of the megagametophyte and embryo sac. Such acceleration continues for a period of 17 days after full bloom. At this time the development of the ovary is abruptly arrested. The nucellus and



FIGS. 11-25.—Camera lucida drawings of stages in development of typical late-ripening variety of sweet cherry (Downer), which produces a high proportion of viable seed. Days before full bloom: fig. 11, 13 days; fig. 12, 7 days; fig. 13, 3 days. At full bloom: fig. 14. Days after full bloom: fig. 15, 1 day; fig. 16, 4 days; fig. 17, 6 days; fig. 18, 8 days; fig. 19, 13 days; fig. 20, 17 days; fig. 21, 18 days; fig. 22, 21 days; fig. 23, 27 days; fig. 24, 36 days; fig. 25, 67 days.

integuments also slow down in growth, but not so sharply: The endosperm and embryo make no appreciable growth.

During the period of retarded development of the pericarp, namely, from the 17th to the 31st day after full bloom, there is a sudden acceleration in growth of the endosperm and embryo. A second period of rapid pericarp development follows the period of retarded development, extending from the 31st day after full bloom to fruit ripening. At this time the nucellus and integuments reach maximum size. The endosperm and embryo continue their development, although at a slower rate. The end result is a ripe fruit containing a viable seed.

Development of an early-ripening variety

For the purpose of tracing the development of an early-ripening sweet cherry, the Early Purple Guigne was selected. This variety is typical of early-ripening sorts which normally produce few or no viable seeds.

1. OVARY.—The ovary increases in length only slightly from the 40th to the 14th day before full bloom. At this time it begins a more rapid period of enlargement, until at the time of full bloom it is 1.94 mm. long.

At the time of fertilization there is a sudden and very rapid acceleration in development of the pericarp which continues for a period of 17 days after full bloom. During the season of 1931 the ovary increased in length from 2.01 mm. the day before full bloom to 2.68 mm. the day following. It measured 3.80 mm. the 5th day after bloom, 4.87 mm. on the 7th, 6.75 mm. on the 9th, 8.5 mm. on the 12th, and 12.5 mm. on the 17th. At the end of this period, development of the pericarp ceases almost as abruptly as it began and a period of greatly lessened growth ensues, which in 1931 extended over a period of 8 days, from the 17th to the 25th day following full bloom. The stony pericarp was becoming brittle at the end of this period, and the fruit was beginning to color.

A second period of rapid pericarp development follows the period of retarded development, commencing on the 25th day after full bloom and continuing until the fruit is ripe, namely, the 37th day after full bloom during the season of 1931.

2. **NUCELLUS AND INTEGUMENTS.**—Both ovules develop equally but slowly up to 14 days before full bloom, after which there is a more rapid enlargement, and they double in length by full bloom, unless arrested in development.

At the time of fertilization there is a sudden acceleration in growth of the nucellus and integuments. By 17 days after bloom they have reached a length of 5.85 mm. compared with a maximum of 6.96 mm., reached 26 days after full bloom. At various stages, beginning at this time, the nucellar tissue suddenly collapses and the integuments shrivel and collapse. There is no apparent structural abnormality prior to this collapse, with the possible exception of the region of contact between the nucellus and the developing cellular endosperm. At this point there is a loss of turgor in the nucellus just prior to the general collapse of the nucellus and integuments. The phenomenon suggests a sudden decrease in turgor in the thin-walled cells of the nucellar tissue. In this variety seeds are rarely found with integuments which have not collapsed.

3. **MEGAGAMETOPHYTE AND EMBRYO SAC.**—Development of the megagametophyte and embryo sac is similar in every way to that of the normal variety already described. It progresses regularly and uniformly, no abnormalities being disclosed by a critical study.

4. **FERTILIZATION.**—Pollination and fertilization are as outlined for the variety already described. Pollen tubes were traced through the style, within the ovary and within the micropyle, in the case of both open-pollinated and also emasculated flowers pollinated by hand. Tested pollen was used of known compatible varieties, as already discussed.

5. **ENDOSPERM.**—Development of the endosperm following fertilization progresses similarly to that already described. It is free-nuclear until the 9th day after full bloom, at which time the first cell walls appear. Twelve days after full bloom the cellular endosperm has reached a length of only 0.17 mm. By the 22nd day after full bloom, however, a period of rapid acceleration has begun, during which the cellular endosperm reaches a length of 3.73 mm. 28 days after full bloom, and 5.6 mm. 37 days after full bloom.

At various stages from the 28th day after full bloom until fruit ripening, there occurs a sudden arrest in development of endosperm

formation. Structurally there is no apparent change prior to the time of suspended development.

6. EMBRYO.—The embryo also develops similarly to the previous variety. It develops very slowly following the first division of the zygote, until by the 22nd day after full bloom it has reached a length of only 0.25 mm. At this time it begins a sudden and rapid enlargement, so that in some individuals a length of 1.48 mm. has been reached 28 days after full bloom and 4.42 mm. 32 days after full bloom.

Throughout this period of rapid enlargement, however, there is a sudden cessation of growth in various individuals beginning 25 days after bloom, when the embryos are 0.52 mm. in length. Embryos may be arrested in development at any stage (figs. 6, 7). Structurally there is no apparent change in the embryo prior to abortion. The embryo seems merely to be checked in development during its period of most rapid growth. A similar situation has been reported by DORSEY (6) for the plum.

The aborted embryos often develop a peculiar shape, however, as shown in figures 6 and 7. They are often flattened at the tips of the cotyledons, and one tip is frequently curled over the other. It is suggestive of the flattening of the cotyledons against the arrested endosperm and the collapsed nucellus and integuments, as though the embryo had continued its growth for a period after the initial check in development. BRADBURY reported that the embryos of dropped cherries continued their development for some time later. It must be remembered, however, that she was dealing with aborted embryos in dropped fruits, whereas these results deal with aborted embryos in developing fruits.

There is a general grouping of aborted embryos into four waves, not unlike the waves of dropping fruits reported by BRADBURY. One occurs when the embryo has reached a length of approximately 2.9 mm., another when a group of embryos has reached 3.64 mm., again at a stage of 4.20 mm., and again at 5.73 mm. These groups are not absolute, but mark the points of greatest frequency. The writer has found no viable seed from this variety from several thousands that have been examined over a period of several years. The aborted embryos may begin to disintegrate within 5 days, or they may continue in a viable condition for at least 42 days.

7. CORRELATION OF DEVELOPMENT.—Development of the various parts in relation to one another is shown graphically in figure 10. In addition, figures 26 to 40 present development at given stages as camera lucida drawings.

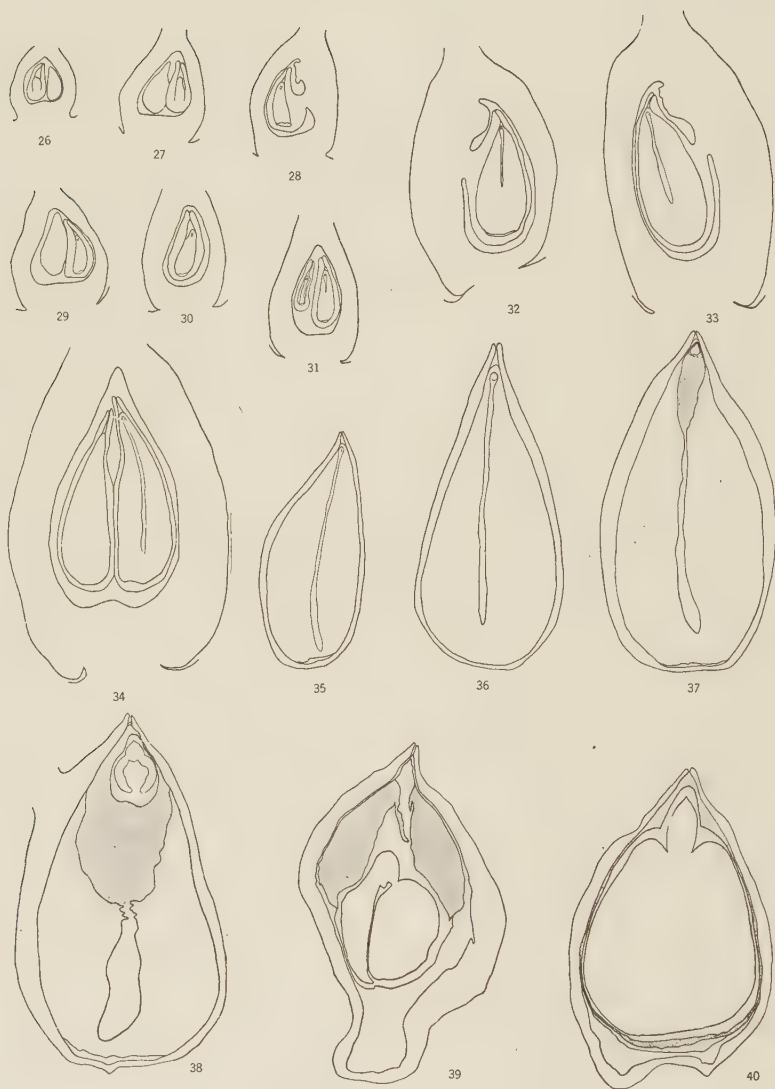
Briefly stated, the ovary and ovule parallel each other in development in the early stages. They increase only slightly in size between the 40th and 14th day prior to full bloom. Between then and the time of full bloom the ovary and nucellus and integuments begin a more rapid enlargement. The megaspore mother cell is formed at the beginning of this period, and the megagametophyte is in turn formed from it 3 to 7 days before full bloom. One of the two ovules is arrested in development just before full bloom, and the other takes the lead.

At the time of fertilization there is a sudden acceleration in growth of the ovary and of the nucellus and integuments, and a marked elongation of the megagametophyte or embryo sac. Such acceleration continues for a period of 17 days after full bloom. At this time pericarp development is abruptly arrested. The nucellus and integuments also slow down in growth but not so sharply. The endosperm and embryo make no appreciable growth. During the period of retarded pericarp development, beginning on the 22nd day after full bloom, there is a sudden acceleration in growth of the endosperm and of the embryo.

The second period of rapid pericarp development, which follows the period of retarded development, extends from the 25th day after full bloom until the fruit is ripe on the 37th day. Thus the second period of rapid pericarp development is initiated and the fruit is beginning to color at the time the embryo and endosperm are in their period of most rapid development.

The nucellus and integuments reach maximum size on the 25th day after full bloom. Beginning at this time, and throughout this second period of rapid pericarp development, there is a sudden cessation of both endosperm and embryo development at various stages of development in various individuals.

The pericarp continues its development to the ripening of the fruit. The embryos abort at various stages, and the nucellus and integuments collapse. The end result is a ripe fruit containing a seed with an aborted embryo.



FIGS. 26-40.—Camera lucida drawings of stages in development of typical early-ripening variety of sweet cherry (Early Purple Guigne), which rarely produces viable seed. Days before full bloom: fig. 26, 12 days; fig. 27, 6 days; fig. 28, 2 days. At full bloom: fig. 29. Days after full bloom: fig. 30, 2 days; fig. 31, 4 days; fig. 32, 5 days; fig. 33, 7 days; fig. 34, 9 days; fig. 35, 12 days; fig. 36, 17 days; fig. 37, 22 days; fig. 38, 28 days; fig. 39, 32 days; fig. 40, 37 days.

Comparative development of early and late-ripening varieties

Dividing development into the stages of (1) pre-bloom, (2) pollination and fertilization, (3) first rapid pericarp development, (4) retarded pericarp development, and (5) second rapid pericarp development, the comparison between the early and late-ripening types may be summarized as follows.

During the pre-bloom period, both kinds of varieties develop similarly in all parts. Pollination and fertilization are likewise similar, except that the early-ripening variety develops slightly in advance of and at a slightly greater rate than the late-ripening variety.

During the period of first rapid pericarp development, beginning at the time of fertilization and continuing until 17 days after full bloom, there is also close similarity. During the period of retarded pericarp development the first significant difference between the two types is reached. In the case of the early-ripening variety, this period extends from the 17th to the 25th day after full bloom, or a span of 8 days. In the case of the late-ripening variety the period extends from the 17th until the 31st day, a span of 14 days. The period of rapid embryo and endosperm development begins about the same time for both types, namely, 25 days after bloom.

The period of rapid pericarp development in the early-ripening variety begins at the time rapid embryo and endosperm development are initiated; in the late-ripening variety this period does not begin until 6 days later. That is to say, at the time the second period of rapid pericarp development begins in the early-ripening variety, the cellular endosperm has reached a length of 2.40 mm. and the embryo a length of 0.52 mm. At the time this period begins for the late-ripening variety the cellular endosperm is 4.8 mm. in length and the embryo 3.0 mm.

Furthermore, the increase in pericarp development in the late-ripening variety continues at a much slower rate. It increases 2.6 mm. in 12 days as contrasted with an increase of 4.5 mm. for the first 12 days of this period for the early-ripening variety. Development of the late-ripening variety continues over a longer period of time, until 66 days from full bloom as contrasted with 37 days for the early-ripening variety.

Embryo and endosperm development of the late-ripening variety

is thereafter regular and uniform until a viable seed is produced. During this period embryo and endosperm development of the early-ripening variety is suddenly checked at varying stages of development, and a viable seed is produced only rarely. The nucellus and integuments collapse to give the characteristic shriveled seed of the early-ripening variety as contrasted with the plump, well-filled seed of the late-ripening type.

Results with other varieties

That the situation described for Early Purple Guigne, representing an early-ripening variety, and for Downer, representing a late-

TABLE I
RELATION BETWEEN TIME OF RIPENING OF FRUIT AND VIABILITY
OF SEED OF SWEET CHERRY IN 1927

VARIETY	NO. OF SEEDS	DAYS BETWEEN BLOOMING AND RIPENING	PERCENTAGE VIABLE SEED
Abundance.....	1915	82	94.5
Black Republican.....	3320	80	91.0
Black Tartarian.....	2437	62	7.2
Downer.....	8897	70	98.3
Eagle.....	1850	64	61.9
Early Purple.....	2494	48	00.1
Elton.....	2025	60	00.0
Governor Wood.....	2275	53	7.2
Knight.....	1600	56	00.0
Lambert.....	2220	78	55.0
Mazzard (Geneva).....	4420	86	100.0
Mazzard (Virginia).....	454	Over 80	97.5
Mazzard (Virginia).....	624	Over 80	93.2
Napoleon.....	1809	75	60.1
Oswego.....	3922	95	100.0
Schmidt.....	2396	71	72.8
Windsor.....	1855	79	43.7

ripening kind, is characteristic also of other varieties is shown in table I, taken from a previous publication (18), showing the general relation between time of fruit ripening and viability of seed.

In addition, examination of fresh material during the seasons of 1931 and 1932 concurs with the previous findings. Measurements and critical examination were made under the dissecting microscope at regular intervals during the season. The results are given in table II. They, too, are in general agreement. It will be noted that there

is a tendency for the embryos of the earliest-ripening varieties to abort at an earlier date than those ripening a few days later. Moreover, there is the tendency for the aborted embryos of the earliest-ripening varieties to be smaller than those of varieties which ripen a little later.

TABLE II

RELATION BETWEEN SEED AND FRUIT DEVELOPMENT AND ABORTION
OF EMBRYOS IN SWEET CHERRY IN 1931

VARIETY	NO. OF DAYS BETWEEN FULL BLOOM AND FRUIT RIPENING	LENGTH IN MM. 37 DAYS AFTER BLOOM				NO. OF DAYS FROM FULL BLOOM TO FIRST INDICATION OF EMBRYO ABORTION	PROPORTION OF VIABLE SEED
		OVARY	NUCELLUS AND INTEGUMENTS	CELLULAR ENDOSPERM	EMBRYO		
Burbank.....	48	17	6.8 (collapsed)	4.0	3.1	31	None
Governor Wood...	48	16	6.0 (collapsed)	5.2	4.0	34	None
Lyons.....	49	18	7.2	5.2	4.8	38	None
Knight.....	52	16	6.7 (collapsed)	6.2	5.9	35	None
Elton.....	56	19	7.9	6.0	5.3	37	None
Black Tartarian...	55	15	7.3	5.8	5.2	43	Some
Black Eagle.....	60	14	7.2	5.8	5.2	50	Most
Schmidt.....	64	15	8.0	6.2	5.3	No abortion	Most
Black Republican.	68	15	7.9	6.2	5.9	" "	Most
Mazzard.....	68	9	7.0	5.5	5.3	" "	All
Abundance.....	75	11	6.8	5.0	4.8	" "	All
Oswego.....	77	11	6.9		4.8	" "	All

Discussion

The sudden acceleration in growth of the ovary, the nucellus and integuments, and the megagametophyte or the embryo sac which occurs at the time of fertilization is not necessarily a response to fertilization in itself. Such acceleration may begin 24 hours before pollen tubes can be found within the ovary, and therefore, before fertilization has been accomplished, or it may be delayed until 4 or 5 days after full bloom, at which time a very small embryo can be distinguished. On the other hand, this rapid growth may begin similarly with unfertilized ovules and continue for 7 to 10 days. It may also begin temporarily with ovules containing a normal endosperm development but no embryo, and with ovules containing an embryo but no endosperm.

Further evidence on this point is presented by emasculated and unpollinated flowers. They show a similar sudden and rapid growth of ovary, of nucellus and integuments, and of megagametophyte. The megagametophyte elongates, the synergids and megagamete remain at the micropylar end of the megagametophyte, and the fused polar nuclei maintain a position nearly midway the length. Such a condition is shown in figure 4, 7 days after full bloom. No fertilization has occurred, and the style has abscised. However, no blossoms of this type develop to the ripe fruit.

These facts suggest that the initiation of the period of rapid growth is *coincident with* the time of fertilization, but not necessarily *initiated by* it.

CONNORS (4), in discussing embryo abortion in early-ripening varieties of peaches, says: "The probable cause of abortion is the fact that the enlargement of the drupe takes place at the same time as the final period of development of the embryo, due to a heritable character determining the period of ripening. The flesh becomes mature and ripe and abscission takes place before the development of the embryo is completed."

That the last part of this suggestion does not apply to the cherry varieties under discussion in this paper is shown by the fact that embryo abortion may occur 12 days prior to ripening of the fruit, and that it occurs at different dates in different varieties. KRAUS (8) has called attention to the fact that this arrest in the development of an embryo is also found in the case of certain matings of fowls and in some mammals.

Since there appear to be no histological nor developmental changes prior to embryo abortion in the early-ripening type of sweet cherry, except for the initiation of the second period of rapid pericarp development at the time the embryo and endosperm are making their most rapid growth, attention is naturally focused upon this relation; yet it is not possible to say which is cause and which is effect. Whether the pericarp develops more rapidly in the early-ripening varieties because the embryo aborts, or whether the embryo aborts because of the rapid development of the pericarp, these data give no indication.

As a general proposition, however, from the behavior of plants in

general, one would hazard the opinion that if there is any nutritional balance between embryo development and pericarp development, the abortion of the embryo is the factor which determines the rate and time of the second period of rapid pericarp development rather than the reverse. The suggestion is made that abortion of the embryo may not by itself be responsible for the dropping of a fruit. Possibly quite to the contrary, embryo abortion may be a varietal character which is responsible for the earliness with which some varieties ripen their fruit.

Not only is embryo abortion a characteristic of later waves of dropped fruits, therefore, as pointed out by KRAUS, BRADBURY, DETJEN, and others, but it is also a characteristic of some varieties of fruits which normally ripen their fruit on the tree.

From the standpoint of the plant breeder, the establishment of the fact that early-ripening varieties fail to produce seed with fully developed embryos means that in efforts to breed early-ripening sorts, an early-ripening kind should not be used as the female parent. There is the suggestion from the studies of embryo culture by LAIBACH (9), TUKEY (19), and others that some progress might be made in this direction. By culturing an embryo from an early-ripening type upon which another early-ripening variety had been used as the male parent, the chances would be increased of securing a new individual with a combination of genes for early-ripening from both male and female parent. Once a horticultural variety is produced, its perpetuation is assured by the usual vegetative methods of propagation, such as budding and grafting.

That various parts of the fruit and seed may be developing at one time and other parts at another is an interesting point. Instead of the ovary, the nucellus and integuments, the megagametophyte, the endosperm, and the embryo all developing at similar times with parallel growth rates, they develop at different periods with varying growth rates. In spite of this seeming complexity and variability, the factors governing development are remarkably constant. That is to say, if the size of any one part is known, it is possible to tell the relative development of any related part at that time. In other words, when the embryo is a given size, the endosperm development bears a constant relation to it, the nucellus, integuments, and the

pericarp as well. This applies not only to any one season but to different seasons in comparison with one another. The graphs of development may vary in steepness and length, depending upon the season, but the relation between developing parts is constant regardless of the season.

The period of retarded pericarp development following a second period of rapid development is a characteristic of drupaceous fruits. LILLELAND (10) suggests that the retarded growth may be ascribed to the competition for food substances used in forming the stone. He considers that the fact that this cyclic type of growth has not been found in pome fruits further substantiates this premise.

Factors underlying embryo abortion as determined from cross-breeding records

Some light is shed on the factors underlying embryo abortion by the plant breeding records of the New York State Agricultural Experiment Station. Over a period of 7 years they show a minimum of crosses involving early-ripening varieties of cherries as female parents. This is because practical plant breeders have been rewarded with no progeny from such crosses, and therefore have purely empirically stricken such crosses from their cross-breeding programs. The few crosses that have been made which involve early-ripening varieties, however, show that when such varieties of cherries are used as female parents, no viable seed is produced, even though the male parent in the cross is a late-ripening kind. On the other hand, when late-ripening varieties of sweet cherry are used as female parents, viable seed is produced. The use of an early-ripening variety as male parent does not alter the results.

Table III gives the results from 13 recorded crosses over a period of 7 years involving early-ripening varieties as either male or female parent. The number of days elapsing between full bloom and ripening of fruit is given in parentheses after each variety. Unfortunately, there are only two reciprocal crosses, namely, between Seneca (35) and Black Tartarian (55), and between Burbank (48) and Lyons (49), and both of these crosses involve early or early mid-season varieties. Yet the figures tend to show that the limiting factor in the production of viable seed is the nature of the female parent and not

the male parent, and that the problem concerns physiology and nutrition rather than genetics.

Correlation between ripening of fruit and abortion of ovule and embryo

It has already been indicated that there is a tendency for the embryos of the earliest-ripening varieties to abort at an earlier date than those ripening a few days later, and that there is a tendency for the aborted embryos of the earliest-ripening varieties to be smaller

TABLE III

PERCENTAGE VIABLE SEED SECURED FROM CROSS-BREEDING IN WHICH BOTH
EARLY AND LATE-RIPENING VARIETIES OF CHERRY ARE USED
AS BOTH MALE AND FEMALE PARENTS

FEMALE PARENT	DAYS TO RIPEN FRUIT	MALE PARENT	DAYS TO RIPEN FRUIT	NO. OF FRUITS	PERCENT- AGE VIABLE SEED
Seneca.....	(35)	× Burbank	(48)	2	0
Seneca.....	(35)	× Lyons	(49)	7	0
Seneca.....	(35)	× Black Tartarian	(55)	13	0
Seneca.....	(35)	× Abundance	(75)	13	0
Early Purple Guigne.	(37)	× Burbank	(48)	10	0
Early Purple Guigne.	(37)	× Lyons	(49)	21	0
Burbank.....	(48)	× Lyons	(49)	29	0
Burbank.....	(48)	× Black Tartarian	(55)	12	0
Lyons.....	(49)	× Burbank	(48)	28	0
Black Tartarian.....	(55)	× Seneca	(35)	456	8
Eagle.....	(60)	× Seneca	(35)	33	32
Eagle.....	(60)	× Republican	(68)	38	26
Giant.....	(65)	× Seneca	(35)	7	57

than those of varieties which ripen a little later. The question arises, therefore, as to any possible relation between fruits of varying degrees of ripeness on any one day on any given tree. That is, is there a correlation between accelerated pericarp development and abortion of embryo within the same variety?

In an attempt to answer this question, samples of fruits were collected each day during the season of 1932. The method was to take all the fruits on a given branch for each sampling. From 10 to 60 fruits were taken in each sample, involving a total of several thousand individuals, from which the data in tables IV and V were secured. The fruits from each variety were divided each day into various classes, according to degree of ripening as indicated by color.

It was at once apparent that there was a greater degree of variability in ripening among the early-ripening varieties than among the late-ripening kinds. The fruits of Early Purple Guigne, for example, within a span of 12 to 18 inches on a given twig, could be divided into green, greenish-yellow, yellow, red tinge, and half red.

TABLE IV
RELATION BETWEEN FRUIT RIPENING IN LATE-RIPENING VARIETY OF SWEET
CHERRY AND DEVELOPMENT OF NUCELLUS AND
INTEGUMENTS AND EMBRYO

PROGRESS IN RIPENING	LENGTH IN MILLIMETERS									
	JUNE 19	JUNE 20	JUNE 21	JUNE 22	JUNE 23	JUNE 24	JUNE 25	JUNE 26	JUNE 27	JUNE 28
Total pericarp										
Green.....	12.86		13.02	12.92	12.90	13.34				
Red tinge.....	13.10		13.10	13.16		13.36	13.52	13.82	14.34	
Half red.....					13.82		14.45	14.52		
Full red.....									14.64	14.76
Nucellus and integuments										
Green.....	7.76		7.84	7.72	7.84	7.76				
Red tinge.....	7.84		7.80	7.76		7.76	7.96	8.00	7.56	
Half red.....					8.12		8.21	8.00		
Full red.....									7.82	7.76
Embryo										
Green.....	6.52		6.88	6.94	6.80	7.04				
Red tinge.....	6.80		6.70	6.90		6.96	6.60	7.12	6.84	
Half red.....					7.00		7.30	7.28		
Full red.....									7.04	7.12

On the other hand the fruits of Downer, on a given twig, were more uniform in development; that is, nearly all either in the green stage, the greenish-yellow stage, or the yellow stage, and so on. It was only with difficulty that various degrees of ripening could be secured from the late-ripening varieties of cherry, like Downer.

Likewise it was at once apparent from dissections that there was greater variation in size of embryo, endosperm, and nucellus and

integuments within the early-ripening varieties than in the late-ripening ones. The latter were strikingly similar in size and development on any given date.

TABLE V

RELATION BETWEEN FRUIT RIPENING IN EARLY-RIPENING VARIETY OF SWEET CHERRY AND DEVELOPMENT OF NUCELLUS AND INTEGUMENTS AND EMBRYO

PROGRESS IN RIPENING	LENGTH IN MILLIMETERS										
	JUNE 5	JUNE 6	JUNE 7	JUNE 8	JUNE 9	JUNE 10	JUNE 11	JUNE 12	JUNE 13	JUNE 14	JUNE 15
Pericarp											
Green.....	13.58	13.48									
Red tinge.....	13.77	13.81	14.40	14.73		14.40					
Half red.....		14.70	15.13	15.23		14.64	15.32				
Three-fourths red.....	14.22	15.26	15.80		15.10	14.86	15.56	15.88		16.22	
Full red.....	14.30				15.56		15.60	16.03		16.41	17.16
Nucellus and integuments											
Green.....	7.15	7.38									
Red tinge.....	6.91	7.13	6.80	6.56		7.00					
Half red.....		6.42	6.53	6.40		6.40	6.96				
Three-fourths red.....	6.36	6.40	6.30		6.73	6.43	6.56	6.88		6.84	
Full red.....	5.60				6.60		6.10	6.66		6.46	6.96
Embryo											
Green.....	3.41	3.66									
Red tinge.....	3.47	3.74	3.52	3.16		4.65					
Half red.....		3.06	2.90	3.90	4.96	4.68	5.54				
Three-fourths red.....	2.90	3.63	3.70	3.48	4.73	5.13	5.08	5.44		5.68	
Full red.....	1.60				4.58		3.83	5.63		5.76	6.04

As might be expected, the larger fruits on any given date were farthest along in ripening, as shown in tables IV and V and also in figure 41. Yet all flowers bloomed within a span of 2 days, so that it is not possible to lay this difference to lateness in blooming of some flowers as compared with others on the same branch or twig.

Variation in time of pollination or in time of fertilization could be responsible for the variation; but if this is so, it is difficult to understand why the fruits of a variety like Downer developed uniformly, while those of a variety like Early Purple Guigne developed irregularly.

An examination of the nucellus and integuments of the late-ripening variety, Downer, shows this same relation. Larger nucellus and

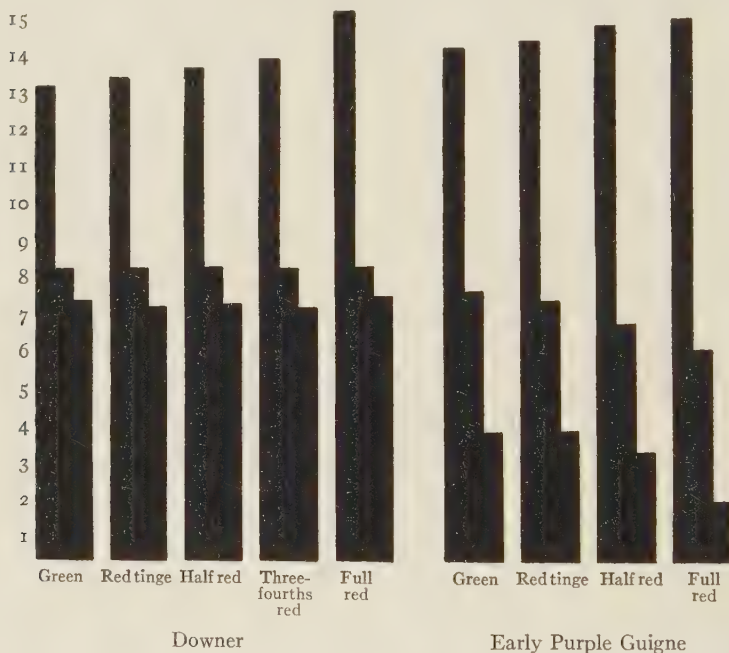


FIG. 41.—Relation between size of pericarp and embryo and nucellus and integuments in fruits of different degrees of maturity picked on the same day. In each column, left-hand section represents the pericarp, center section the nucellus and integuments, and right-hand section the embryo.

integuments are found with larger and riper fruits (table IV). On the other hand, the early-ripening variety, Early Purple Guigne, shows an exactly inverse relation. The largest and most nearly ripe fruits on any given date have the smallest ovules (table V). This fact is most readily seen in figure 41.

Likewise with the embryos, the largest embryos are found in the

largest and ripest fruits of the late-ripening variety, Downer, on any given date, thus correlating large fruit-size and advanced ripening with large size of nucellus and integuments and large size of embryo. And here again, in the case of the early-ripening variety, Early Purple Guigne, the relation is inverse. The smallest embryos are found in the largest and ripest fruits collected on any one day. Yet this inverse relation is not so regular as in the case of the nucellus and integuments. The embryos are often larger, proportionately, than the nucellus and integuments. This suggests that an embryo may continue development for a time independently of development of the nucellus and integuments, as BRADBURY has indicated in abscissed fruits of sour cherry. Figure 8 also shows an embryo which has developed while still contained within the integuments of an abortive seed, in this case producing six true leaves.

The inference to be drawn from these facts is that a check in the development of the nucellus and integuments precedes a check in the development of the embryo. Furthermore, since the embryo seems often to continue development after arrest of other parts of the seed, the underlying cause of embryo abortion is pushed back one step further, namely, to whatever is responsible for cessation of development of the nucellus and integuments. That this is nutritional seems likely, but not necessarily proved by these data.

There is, however, no light shed on whether the check in development of the ovule is due to rapid development of the pericarp, or whether rapid development of the pericarp is in turn due to an abortion of the ovule and the developing embryo. All that can be said is that in varieties of cherries which normally abort the embryo, the largest and ripest fruits on any one given day normally contain the smallest nucellus and integuments and also the smallest embryos.

Artificial culture of normally abortive cherry embryos

Further evidence that the problem is one of nutrition is given by the artificial culture of cherry embryos (19) which was suggested by the study here reported and by the work of LAIBACH and others on the culture of plant embryos. It was reasoned that since there appeared nothing structurally abnormal in aborting embryos, and since embryos in aborted seeds and fruits often continue to de-

velop for a time after abscission, that if given the proper conditions for growth, these aborting embryos might continue to develop. That this has been the case, and that seedlings with true foliar leaves have been grown by this method from normally aborting embryos, show that lack of proper nutritive conditions rather than any hereditary lethal factor has been responsible for failure of early-ripening varieties to produce viable seed.

Summary

1. Both nurserymen and plant breeders experience difficulty in securing seedlings from seed of early-ripening varieties of sweet cherry (*Prunus avium*).

2. Critical developmental studies are reported for a late-ripening type, the variety Downer, and an early-ripening type, the variety Early Purple Guigne.

3. Comparison is made between the development of these two varieties during the seasons of 1930, 1931, and 1932, and also with other varieties: Burbank, Governor Wood, Lyons, Knight, Elton, Black Tartarian, Black Eagle, Schmidt, Napoleon, Black Republican, Abundance, Oswego, and Mazzard.

4. The embryogeny of the sweet cherry is essentially the same as described by BRADBURY for the sour cherry, and as described by PÉCHOUTRE, RUEHLE, and others.

5. The megaspore mother cell is distinguished 10 to 14 days before full bloom, at about the time the flower blossoms have emerged from the bud and have just separated in the cluster. Reduction division occurs several days later. The megagametophyte is formed 3 to 7 days before full bloom, about when the petals are showing.

6. The ovule lengthens but slightly between the 55th day before full bloom and the time of full bloom. The non-functional ovule is arrested in development just prior to full bloom, but the megagametophyte in the aborted ovule may persist unelongated, but otherwise complete, for 10 to 14 days longer.

7. Ovary development parallels that of the nucellus, in which increase in length is slight from the 55th day before full bloom until full bloom.

8. At the time of fertilization there is a sudden acceleration in

growth of the ovary and of the nucellus and integuments, and a marked elongation of the megagametophyte or embryo sac, which continues for a period of 17 days after full bloom. The nucellus and integuments reach nearly maximum size, and the embryo sac reaches nearly to the chalaza.

9. Development of the embryo and endosperm is much delayed. The endosperm remains free-nuclear until the 9th day after full bloom, and is then cellular only for a short distance. Subsequent development is by cell division progressively from the micropylar end toward the chalazal end, and from the periphery toward the center. The free-nuclear endosperm in the chalazal end of the embryo sac does not become cellular.

10. Seventeen days after bloom rapid pericarp development ceases almost as abruptly as it begins and a period of retarded growth follows. The period of retarded pericarp development continues 8 days for the early-ripening variety and 14 days for the late-ripening kind. During this period the embryo and cellular endosperm begin a sudden and rapid development.

11. A second period of rapid pericarp development follows the period of retarded development. It begins 31 days after full bloom for the late-ripening type and 25 days after bloom for the early-ripening type. The embryo of the late-ripening type has developed to a length of 3 mm., whereas the embryo of the early-ripening type is only 0.52 mm. in length.

12. The embryo of the late-ripening variety during this period develops to maximum size, surrounded by a thin layer of endosperm and a line of nucellar tissue, all contained within the two integuments, to give the viable seed characteristic of this type of fruit. On the other hand, in the case of the early-ripening variety, four waves of embryo abortion occur at this time. The nucellar tissue collapses, the integuments shrivel, and the characteristic abortive seeds of early-ripening varieties are the result.

13. Embryo abortion is observed as a sudden arrest in development of the embryo. No apparent structural variation has been observed in abortive embryos, with the exception of the tendency for such embryos to assume a flattened shape at the tips of the cotyledons, with the cotyledons overlapping each other.

14. The first indication of irregularity in development occurs at the region of contact between the nucellar tissue and the developing cellular endosperm. At this point there is a loss of turgor in the nucellar tissue just prior to the general collapse of the nucellus and integuments.

15. Although abortive embryos are frequently associated with dropped fruits, in this case they are associated with normally ripening fruits. Abortive embryos need not necessarily be associated with poor pollination, sterility, incompatibilities, or even nutrition.

16. Whether the pericarp develops more rapidly in the early-ripening varieties because the embryo aborts, or whether the embryo aborts because of the rapid development of the pericarp, these data give no indication. Although all parts do not develop at the same rates and during the same periods, yet they bear the same relation to one another even in different years.

17. The sudden acceleration of parts at the time of fertilization is coincident with the time of fertilization but not necessarily initiated by it. Emasculated and unpollinated flowers show the same sudden rapid development of ovary and of nucellus and integuments, and the marked elongation of the megagametophyte as in the case of hand-pollinated and fertilized blossoms, but do not continue to the formation of ripe fruit.

18. The effect of emasculation and bagging is to retard the development of all flower parts by from 4 to 6 days.

19. In the central zone of the nucellus, reaching from the chalaza to the base of the megaspore mother cell and the megagametophyte, a core of elongated cells is formed which suggests a conductive system, and which is the tissue through which the megagametophyte and embryo sac elongate in their subsequent development.

20. Early-ripening varieties of sweet cherry should not be used as female parents in the breeding of new varieties. Breeding records involving late-ripening and early-ripening varieties of cherry show no progeny when early-ripening varieties are used as female parents, but do show progeny when used as male parents, indicating that the problem of production of non-viable seed is not genetic.

21. Among varieties of cherries which normally contain abortive embryos, the earliest-ripening ones contain the smallest embryos.

22. The largest and ripest fruits on a given branch of a late-ripening variety on any one day contain the largest ovules and embryos. This relation is exactly the reverse for early-ripening varieties, in which the ovules and embryos are smallest in the earliest-ripening fruits. These facts indicate that a check in the development of the nucellus and integuments precedes a check in the development of the embryo.

23. Further evidence that the problem is one of nutrition is given by successful artificial culture of normally abortive embryos.

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